

Listing of the Claims:

Claim 1. (Withdrawn) Novel thrombolytic enzyme named Thrombinase having a molecular weight in the range of 31,000 to 32000 useful for dissolving blood clots.

Claim 2. (Withdrawn) Novel thrombolytic enzyme named Thrombinase as claimed in claim 1 having a molecular weight of 31700 useful for dissolving blood clots.

Claim 3. (Currently amended) A process for preparation of thrombolytic enzyme, named as Thrombinase having a molecular weight in the range of 31000 to 32000 Daltons which comprises

- (i) ~~Culturing~~ culturing the filtrate of *Bacillus sphaericus* serotype H5a 5b in a culture medium consisting of yeast extract with one or more of constituents selected from peptone, sodium acetate, beef extract, sodium chloride, Soya peptone, and ammonium sulphate,
- (ii) ~~Removing the cell formed by cross flow filtration using 0.22 μ filter~~
separating cells, from a cell supernatant, in cross flow filtration using a 0.22 μ filter,
- (iii) ~~Subjecting~~ subjecting the cell supernatant thus obtained to two step ultra filtration using a cut off membrane of at least 100,000 ~~1,00,000~~ MW (Molecular Weight) cut off membrane followed by ultra filtration of the filtrate thus obtained using 10,000 MW cut off membrane,
- (iv) ~~Salting~~ salting out the retentate with ammonium sulphate,

- (v) ~~Subjecting~~ subjecting the resulting precipitate to dialysis,
- (vi) ~~Re-precipitating~~ re-precipitating the precipitate using ice-cold acetone,
- (vii) ~~Reconstituting~~ reconstituting in buffer,
- (viii) ~~Decolorizing~~ decolorizing by using modified CDR (Cell Debris Remover) treatment, dialyzing, lyophilizing,
- (ix) ~~Purifying~~ purifying firstly by ion exchange chromatography followed by gel filtration chromatography and
- (x) ~~Dialyzing~~ dialyzing the fraction showing fibrinolytic activity and lyophilizing ~~to obtain and obtaining~~ purified Thrombinase having a molecular weight in the range of 31,000 to 32000 Daltons.

Claim 4. (Original) A process as claimed in claim 3 wherein the amount of the constituents present in the Culture medium employed is 0.03 to 1.5% of yeast extract, 0.2 to 1.5% peptone, 1 to 1.6% sodium acetate, 0.3 to 0.5% beef extract, 0.2 to 0.5% sodium chloride, 0.5 to 1% Soya peptone, and 0.68% ammonium sulphate.

Claim 5. (Previously presented) A process as claimed in claim 3 wherein the pH of the culture medium used is in the range of 7.2 to 8.0.

Claim 6. (Previously presented) A process as claimed in claim 3 wherein the amount of ammonium sulphate used is in the range of 20 to 40%.

Claim 7. (Previously presented) A process as claimed in claim 3 wherein the buffer used is Tris 0.01 M and the pH is 8.0.

Claim 8. ((Previously presented) A process as claimed in claim 3 wherein the amount of ice-cold acetone and crude enzyme used are in the ratio of 1:1 to 1:1.5 (v/v).

Claim 9. (Withdrawn) The use of the novel enzyme named Thrombinase having a molecular weight in the range of 31,000 to 32000 for dissolving blood clots.

Claim 10. (Cancelled).

Claim 11. (New) A process as claimed in claim 3 wherein the amount of the constituents present in the Culture medium employed is 0.03 to 1.5% of yeast extract, 0.2 to 1.5% peptone, 1 to 1.6% sodium acetate, 0.3 to 0.5% beef extract, 0.2 to 0.5% sodium chloride, 0.5 to 1% Soya peptone, and 0.68% ammonium sulphate; wherein the pH of the culture medium used is in the range of 7.2 to 8.0, whereby the product of (x) has the capacity for dissolving blood clots.

Claim 12. (New) A process as claimed in claim 11 wherein the amount of ammonium sulphate used is in the range of 20 to 40%.

Claim 13. (New) A process as claimed in claim 12 wherein the buffer used is Tris 0.01 M and the pH is 8.0.

Claim 14. (New) A process as claimed in claim 13 wherein the amount of ice-cold acetone and crude enzyme used are in the ratio of 1:1 to 1:1.5 (v/v).

Claim 15. (New) A process as claimed in Claim 3, wherein the purified thrombinase has a molecular weight in the range of 31,700 Daltons.

Claim 16. (New) A process as claimed in Claim 11, wherein the purified thrombinase has a molecular weight in the range of 31,700 Daltons.

Claim 17. (New) A process as claimed in Claim 12, wherein the purified thrombinase has a molecular weight in the range of 31,700 Daltons.

Claim 18. (New) A process as claimed in Claim 13, wherein the purified thrombinase has a molecular weight in the range of 31,700 Daltons.

Claim 19. (New) A process as claimed in Claim 14, wherein the purified thrombinase has a molecular weight in the range of 31,700 Daltons.